AMENDMENTS TO THE SPECIFICATION

The following is a marked-up version of the specification with the language that is underlined ("___") being added and the language that contains strikethrough ("—") being deleted:

The HEADER section:

Top of each page:

[50508-2390] 50508-1390

The BACKGROUND section:

(paragraph starting at page 1, line 30)

Successful fertilization in mammals is dependent upon the species-specific recognition, adhesion, and fusion between sperm and egg. Despite their fundamental importance, little is known about the molecular basis underlying these events. Two sperm-egg recognition events in particular have received the most attention: the initial adhesion between the sperm plasma membrane and the egg extracellular coat, or zona pellucida, and the binding between membranes of the acrosome-reacted sperm and the egg plasma membrane ([Prima-koff] Primakoff and Myles, 2002; Wassarman et al., 2001). In both instances, candidate receptors have been identified, but thus far, none of these receptors appear to be completely responsible for either sperm-egg binding or sperm-egg fusion (Miller et al., 1992; Nishimura et al., 2001; Rankin et al., 1998). In particular, sperm binding to the zona pellucida is thought to involve recognition of specific glycoside residues on the ZP3 glycoprotein (Florman and Wassarman, 1985), which lead to aggregation of the sperm receptor and trigger acrosomal exocytosis. The nature of the sperm binding oligosaccharides on ZP3 remains unclear, as are the sperm proteins that bind ZP3 (Florman and Wassarman, 1985; Johnston et al., 1998; Miller et al., 1992; Nagdas et at., 1994; Nishimura et al., 2001; Primakoff and Myles, 2002; Rankin etal., 1998; Wassarman et al., 2001).

The SUMMARY section:

(paragraph starting at page 3, line 1)

The disclosure generally provides methods and compositions for modulating mammalian gamete adhesion. The methods and compositions may be used for both diagnostic and therapeutic purposes. Representative modulators of gamete adhesion include, but are not limited to, polypeptides comprising at least one discoidin/C domain, and optionally at least one EGF domain. A representative polypeptide includes, but is not limited to, SED1 polypeptides (SEQ ID ~~NO.~~ NOs. 2-7), prodrugs, fragments, or derivatives thereof.

The BRIEF DESCRIPTION OF THE FIGURES section:

(paragraph starting at page 4, line 16)

FIG. 3D is an immunoblot showing SED1 binds to the area corresponding to ZP1/ZP2 and to ZP3[ ]. Similarly, when ZP blots were probed with SED1 expressed either in COS-7 cells (SED1-COS) or in bacteria (rSED1) and detected with α-SED1 antibody, binding to ZP1/ZP2 and ZP3 under reducing conditions is seen.

(paragraph starting at page 5, line 8)

FIG. 6A is an immunoblot showing the presence or absence of SED1 and ~~GalT~~ GalT in sperm from wild type and SED1 null mice.

(paragraph starting at page 5, line 17)

FIG. 7A and 7B show diagrams of exemplary ~~Models~~ models for SED1 function during sperm-egg binding.

The DETAILED DESCRIPTION section:

(paragraph starting at page 5, line 24)

It has been discovered that SED1 functions during mammalian fertilization. Mammalian fertilization was investigated using the mouse model. SED1 was cloned from mouse testis and denotes a secreted protein containing N-terminal Notch-like type II EGF repeats and C-terminal discoidin/F5/8 Complement domains (also referred to as discoidin C domains or discoidin/F5/F8 C domains). Murine SED1 is localized in the Golgi complex of differentiating spermatogenic cells, as well as in the initial segment of the caput epididymis, which together culminate in SED1 being tightly associated with a discrete domain of the sperm plasma membrane overlying the acrosome. Recombinant SED1 binds selectively to the zona pellucida of unfertilized oocytes, and competition assays with recombinant SED1, various SED1 domain constructs, and anti-SED1 antibodies indicate that SED1 participates in sperm-egg adhesion. SED1 null males show greatly reduced [ ]fertility in vivo, and their sperm are unable to bind to the egg zona pellucida in vitro without apparent effects on sperm morphology, number, acrosomal status, or motility. The various embodiments of the disclosure will be discussed in more detail below.

(paragraph starting at page 6, line 12)

The term "[ ]gamete adhesion modulator" refers to a compound that interferes with or promotes the adhesion of a male gamete to a female gamete. A gamete adhesion modulator includes, but is not limited to, polypeptides, small molecules, organic molecules, heterocyclic compounds, aromatic compounds, and the like. An exemplary gamete adhesion modulator includes, but is not limited to, SED1 polypeptides and polypeptides comprising at least one discoidin/C domain.

(paragraph starting at page 14, line 26)

Another method provides combining mammalian sperm and a mammalian unfertilized oocyte in the presence of an amount of a polypeptide effective to modulate gamete adhesion, wherein the polypeptide includes at least on discoidin/C domain. The polypeptide optionally includes at least one EGF domain. In practice,

the polypeptide competitively inhibits in vivo or in vitro binding of sperm to unfertilized zona pellucida or promotes in vivo or in vitro binding of sperm to unfertilized zona pellucida. A representative polypeptide[,] includes, but is not limited to, SED1 polypeptide or a fragment thereof and SEQ ID NOs. 2-7 or a fragment thereof.

(paragraph starting at page 15, line 31)

Yet another embodiment provides a contraceptive method. A representative contraceptive method includes contacting a mammalian gamete with a compound that competitively interferes with SED1-mediated gamete adhesion. The compound can be a polypeptide, for example SED1 polypeptide (SEQ ID ~~NO.~~ NOs. 2-7) or an antibody specific for SED1 polypeptides. Generally, the compound includes a polypeptide comprising at least one discoidin/C domain and optionally comprises at least one EGF domain.

(paragraph starting at page 25, line 23)

Certain examples of prokaryotic hosts are *E. coli* strain RR1, *E. coli* LE392, *E. coli* B, *E. coli* [X] χ[ ]1776 (ATCC No. 31537) as well as *E. coli* W3110 (F-, lambda[-]-, prototrophic, ATCC No. 273325); bacilli such as *Bacillus subtilis*; and other enterobacteriaceae such as *Salmonella typhimurium*, *Serratia marcescens*, and various *Pseudomonas* species.

(paragraph starting at page 31, line 5)

Various techniques suitable for use in protein purification will be well known to those of skill in the art. These include, for example, precipitation with ammonium ~~sulphate~~ sulfate, polyethylene glycol, antibodies and the like or by heat denaturation, followed by centrifugation; chromatography steps such as ion exchange, gel filtration, reverse phase, hydroxylapatite and affinity chromatography; isoelectric focusing; gel electrophoresis; and combinations of such and other techniques. As is generally known in the art, it is believed that the order of conducting the various

purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified protein or peptide.

(paragraph starting at page 31, line 32)

Pharmaceutical compositions and dosage forms of the disclosure include a pharmaceutically acceptable salt of disclosed compositions or ~~compunds~~ compounds or a pharmaceutically acceptable polymorph, solvate, hydrate, dehydrate, co-crystal, anhydrous, or amorphous form thereof. Specific salts of disclosed compounds include, but are not limited to, sodium, lithium, potassium salts, and hydrates thereof.

(paragraph starting at page 36, line 18)

Full-length SED1 with a N-terminal GST tag was constructed using the pMelBac baculovirus transfer vector (Invitrogen). Upon [cotrans-fection] cotransfection with Bac-N-Blue DNA (Invitrogen), viral DNA containing SED1 was isolated, and high-titer viral stocks were produced. High five cells were infected and incubated for 65 hr at 27°C, and the cell supernatant was assayed for SED1 expression by Western blotting. For GST-SED1 purification, the cell supernatant was adjusted to pH 7.4 and 1 M urea (final concentration) with subsequent addition of glutathione-Sepharose (Pharmacia). GST-SED1 was eluted from glutathione-Sepharose using 100 mM phosphate (pH 8.6), 50 mM glutathione, 1 M urea, and 1 mM DTT, followed by extensive dialysis in PBS with 0.3 mM DTT.

(paragraph starting at page 37, line 11)

For Western blot analysis, cauda epididymal sperm were washed, pelleted, solubilized in 8 M urea, and the soluble sperm proteins collected after centrifugation. SED1-expressing SF9 cells were washed free of medium, sonicated ,and centrifuged to remove insoluble material. Mouse milk was directly solubilized in sample buffer. In all instances, soluble proteins were resolved on 10% [polyacryl-

amide] polyacrylamide gels and transferred onto Immobilon-P membranes ([Millipore] Millipore). The membranes were blocked in TBST (150 mM NaCl, 50 mM Tris/HCl [pH 7.5], and 0.1 % Tween 20) containing 2% BSA for one hour at 25°C. After incubation with anti-SED1 or anti-GalT I (Youakim et al., 1994) antibody and rinsing, the bound IgG was detected with goat anti-rabbit-HRP (Amersham) and developed using ECL+plus (Amersham). Protein loading was assayed by probing blots with monoclonal antibodies against [a-tubulin] α-tubulin (Sigma: clone B-5-1-2).

(paragraph starting at page 39, line 2)

[Neutr-Avidin] NeutrAvidin-labeled fluorescent beads (Fluo-Spheres, Molecular Probes), 1 µm, were coated with biotinylated SED1 proteins as described by the manufacturer. WGA-biotin served as a positive control protein; UEAI-biotin, BSA-biotin, cytochrome C-biotin, and uncoupled Fluo-Spheres were used as negative controls (Aviles et al., 1997). Ovulated oocytes, two-cell embryos, and zona pellucida from ovarian oocytes were isolated as described and combined into 40 µl droplets of dmKBRT. To these droplets, 5-10 µl of protein-coated Fluo-Spheres were added and incubated for 1 hr at 25°C. The suspension was washed with a microbore pipette to remove unbound Fluo-Spheres and subsequently examined under the microscope.

The EXAMPLES section:

(paragraph starting at page 43, line 14)

Intact $(His)_6$-SED1 containing both EGF repeats both [dIscoidin/C] discoidin/C domains (EECC) inhibited sperm-egg binding, as did truncated forms of SED1 (EEC, ECC, EC, CC). Furthermore, the intact and truncated SED1 proteins produced similar levels of inhibition, at constant molar ratios, as long as one discoidin/C domain was present (Figure 2C). Thus, EGF domains are not required for competitive inhibition of sperm-egg binding, implicating the discoidin/C domains in mediating SED1 binding to gamete surfaces. This possibility is consistent with

structural and biochemical data, indicating that the discoidin/C domains are able to bind a variety of molecular species, including phospholipid headgroups on membranes, such as on sperm, as well as carbohydrate epitopes in the extracellular matrix, as would be present in the zona pellucida (Fuentes-Prior et al., 2002; Macedo-Ribeiro et al., 1999; Pratt et al., 1999).

(paragraph starting at page 44, line 6)

The results from competitive inhibition assays above are consistent with SED1 participation in sperm-egg binding and implicate the discoidin/C domains in mediating SED1 attachment to gametes. Direct binding of full-length SED1, as well as SED1 truncated proteins, to the zona pellucida was investigated by assaying (1) the binding of SED1[ ]-conjugated beads to intact zona pellucida, and (2) the binding of recombinant SED1 to distinct zona pellucida glycoproteins resolved by SDS-PAGE.

(paragraph starting at page 44, line 13)

SED1-conjugated 1 µm fluorescent beads were added to droplets containing three sources of zona pellucida: zona pellucida fragments isolated from ovaries; intact, unfertilized ovulated oocytes; and two-cell embryos. Any nonadherent beads were removed by washing. SED1-conjugated fluorescent beads bound directly to the zona pellucida of unfertilized oocytes, as well as to zona fragments isolated from ovaries (not shown), but not to the zona pellucida of fertilized two-cell embryos (Figure 3A). Beads conjugated with truncated forms of SED1 also bound to the oocyte zona pellucida, with constructs containing two discoidin/C domains showing higher binding than did constructs with only one discoidin/C domain (compare EECC and ECC versus EEC and EC, Figure 3B). However, the nature of the assay prevented strict quantification of the binding affinities for the different truncated constructs. In any event, these results further implicate the discoidin/C domains in mediating attachment to the gamete surfaces. Consistent with this, the addition of RGD or RAD peptides had no effect on SED1 binding to unfertilized oocytes (not shown). As expected, WGA beads bound to unfertilized zona pellucida similar to SED1 beads, whereas control beads bound at background levels. Controls included

unconjugated beads, as well as beads conjugated with Ulex europaeus agglutinin-1 (UEA1) lectin, BSA, or [cyto-chrome] cytochrome C (data shown for UEA1-lectin, Figure 4C).

(paragraph starting at page 44, line 32)

To determine which of the three zona glycoproteins could serve as the putative ligand for SED1, ovarian, and incubated with [re-combinant] recombinant SED1. When zona glycoproteins were resolved under reducing conditions, SED1 bound to glycoproteins comigrating as ZP1/ZP2 as well as ZP3 (Figure 3D). Similar results were achieved using either biotinylated $(His)_6$-SED1 (D-SED1) detected by Streptavidin-HRP/ECL or recombinant $(His)_6$-SED1 (rSED1) detected by rabbit-anti-SED1 IgG and HRP-conjugated secondary antibodies (Figure 3D). To avoid misleading interpretations due to the use of a bacterially expressed SED1, SED1 was expressed in COS-7 cells, which release budded membrane vesicles that are highly enriched for SED1 (Oshima et al., 2002). Similar to that seen with bacterially expressed SED1, COS-expressed SED1 (SED1-COS) bound ZP1/ZP2 and ZP3 under reducing conditions (Figure 3D).

(paragraph starting at page 47, line 26)

The results presented here support the involvement of SED1 in mammalian gamete recognition and/or adhesion. SED1 is homologous to a small group of bimotif secreted proteins containing N-terminal EGF domains and C-terminal discoidin/complement domains (Andersen et al., 1997, 2000; Couto et al., 1996; Ogura et al., 1996; Stubbs et al., 1990). SED1 is expressed in the Golgi complex of spermatogenic cells and is likely secreted onto the maturing sperm surface. The most abundant expression of SED1 in the male reproductive tract occurs in the initial segment of the epididymis, where sperm are exposed to high levels of secreted SED1. The presence of SED1 immunoreactivity in the adsorptive clear cells of the cauda epididymis suggests that excess SED1 is removed from the sperm environment before they leave the epididymis. On mature sperm, SED1 expression

is confined to the sperm plasma membrane overlying the acrosome, the known location for sperm binding to the egg coat. Recombinant SED1, expressed in either bacteria or insect cells, competitively inhibits sperm-egg binding, as do anti-SED1 antibodies. The biological activity of SED1 requires the discoidin/C domains, which appear to be responsible for SED1 attachment to the sperm membrane and to the zona pellucida matrix. Direct binding of SED1 to the zona pellucida of unfertilized, but not fertilized, eggs is consistent with its role in sperm binding, and immunoblot overlay assays suggest that SED1 recognizes the ZP2 and ZP3 glycoprotein families. Given the ability of discoidin/C domains to bind complex carbohydrates matrices (Fuentes-Prior et al., 2002; [Rei-therman] Reitherman et al., 1975), it is most likely that SED1 is binding to the carbohydrates residues of ZP2 and ZP3.

The EXAMPLES section:

(paragraph starting at page 53, line 10)

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